In vitro antibacterial activities of crude extracts of Nauclea latifolia and Daniella oliveri

*El-Mahmood, A. M, Doughari, J. H. and Chanji, F. J.

Department of Microbiology, School of Pure and Applied Sciences, Federal University of Technology, PMB 2076 Yola 640002, Nigeria.

Accepted 18 February, 2008

The comparative studies of extracts of leaves, barks and roots of Nauclea latifolia and Daniella oliveri with respect to their phytochemical and antibacterial properties was carried out. Preliminary screening for phytochemical components showed that both plants had similar constituents, namely tannins, alkaloids and glycosides, but variable proportions of saponins. The antibacterial screening of the water and ethanolic extracts of the various plant materials were carried out against pathogenic bacteria including Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus and Shigella dysenteriae. Ethanolic extracts were more potent than aqueous extracts and activity were concentration dependent. The Gram positive bacteria were more sensitive to the ethanolic extracts of both plants.

Key words: Nauclea latifolia, Daniella oliveri, phytochemical, antibacterial properties, Gram positive bacteria.

INTRODUCTION

Infectious diseases are the number one causes of death, accounting for approximately one half of all deaths in tropical countries (Iwu et al., 1999). Infact, there are more patients today in hospitals than there are effective drugs due to the development of resistance to available agents. The use of plant parts as a source of medicine to treat infectious diseases predates history. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Erdemeier et al. 1996; Lino and Deogracious, 2006) to cure infections. The intractable problem of antimicrobial resistance has led to the resurgence of interest in herbal products as sources of novel compounds to fight the ever increasing problems of emergence of newer diseases and preventing the resurgence of older diseases thought to be brought under control. Even the World Health Organization (WHO, 2002) is actively encouraging national governments of member countries to utilize their traditional systems of medicines with regulations suitable to their national health care systems. In Nigeria, the Federal Government has urged the federating states to set up traditional medicine boards to license and regulate the practice of herbal practitioners under the supervision of ministries of health.

In this study, we report the finding of a research designed to asses the antimicrobial properties of crude extracts from two selected African medicinal plants that are widely used in Northern Nigeria, both as sources of food (Aiyela`agba et al., 1996), medicine and timber.

MATERIALS AND METHODS

Plant collection and pre-extraction preparation

The plants were chosen based on reports from the local population in Girei town, a superb of Yola, Adamawa state of Nigeria. Voucher specimens were prepared and taken to Federal University of Technology Yola herbarium for identification. The leaves, barks and roots of Nauclea latifolia and Daniella oliveri were collected, sun dried for 7 days, pounded using pestle and wooden mortar.

Extraction procedure

100 g of the ground powder of each of the D. oliveri plant parts were soaked separately in 500 ml of 95% ethanol contained in a 1 L capacity flask for five days. The samples were then strained to remove solids. The solutions were again filtered using Whatman’s filter paper No.1 to obtain a solution free of solids. The solution was then concentrated in a rotary evaporator to remove the ethanol and stored at 2 - 4°C. N. latifolia was extracted in a similar way as D. oliveri. The same procedure was followed for water extracts that 100 g of each sample was dissolved in a 1 l of distilled water, the
solution filtered, solids removed, and then concentrated by drying at 37°C and stored at 2 - 4°C until required.

Preparation of bacteria

The bacteria used were K. pneumoniae, P. aeruginosa, E. coli and S. aureus isolated from clinical specimens obtained from the pathology department of the Federal Medical Centre, Yola. Isolation and identification of the organisms were done following standard procedures in handling clinical specimens (Potashmercher et al., 1979; Cheessbrough et al., 2002). The organisms were maintained on nutrient agar slants at 2 - 8°C. Purity of the organisms was checked at regular intervals by plating and staining (Acheampong et al., 1979). The bacterial cultures were standardized using the method of Baker et al. (1983). The test organisms were suspended into sterile universal bottles containing nutrient broth and normal saline added gradually to it so as to compare the culture turbidity to that of Mc Farland standard, which corresponded to approximately 1.0 x 10^7 cell.ml^-1.

Screening for phytochemical components

The method described by Odebiyi and Sofowora (1978) was used to screen for the presence of the bioactive constituents of the plant materials.

Determination of antibacterial activity

The antibacterial screening was done as described by Lino and Deogracious (2006) and Akoma et al. (2002). Briefly, 1 ml of the test culture (1.0x10^7 cell.ml^-1) was placed into a sterile plate and 19 ml molten agar at 45°C poured and the plate shaken for even spread and proper mixing of the organisms and agar. This agar was allowed to solidify. Four wells of approximately 6 mm in diameter and 2.5 mm deep were made on the surface of the agar plates using a sterile cork borer. The plates were turned upside down and the wells labeled with a marker. The extract was reconstituted by dissolving 1 gm of each aqueous extract in 1 ml of distilled water and each three of the four well was filled with 0.5 ml of extract. The fourth well was filled with 0.5 ml of the pure solvents and served as control and of 12.5 µg equivalent gentamicin was used as a positive control. The plates were incubated at 37°C for 24 h and zones of inhibition measured with a pair of calipers and a millimeter ruler and results tabulated. The same test procedure was repeated for the ethanolic extract. The MIC of the extracts against the test organisms is shown in Table 3 and is similar for both plants. The MIC values ranged from 6.25 to 25 mgml^-1 for K. pneumonia; 50 – 100 mgml^-1 for E. coli; 12.5 -100 mgml^-1 for S. aureus; 6.25 - 50 mgml^-1 for S. dysenteriae and 50 - 100 mgml^-1 P. aeruginosa. The MIC for D. oliveri and are almost half of that of Nauclea latifolia. P. aeruginosa isolates showed less susceptibility to the activity of the crude extract than the other organism. The MIC of gentamicin was less than the crude extracts and ranged between 12.5 to 25 µgml^-1.

Providing effective health care is a challenge under the best of economic circumstances. In the world’s poorest countries, where infectious diseases are rife and resources limited, that challenge can assume overwhelming proportions, hence the resurgence in the use of herbal preparations to treat diseases. In this study, the phytochemical properties of two popular African medicinal trees; D. oliveri and Nauclea latifolia have been assessed for their effects against some clinical isolates of K. pneumoniae, E. coli, P. aeruginosa, S. aureus and S. dysenteriae, obtained from the FMC Yola specialist hospital, a 700 bed tertiary hospital. Each of the 100 g of the dried powdered rootbark yielded a semi solid brown powdery substance of 15 g for the aqueous and 8.6 g for the ethanolic extracts. The leaves, barks and roots of N. latifolia were found to contain alkaloids, tannins and glycosides and traces of saponins, while those of D. oliveri contained alkaloids, tannins, saponins and glycosides as shown in Table 1. The presence of some of the phytochemical components like saponins, tannins and phenolic compounds have been attributed to the antibacterial activity of the crude drugs observed (De and James, 2002). Extracts from the leaves of Eugenia uniflora were found to contain tannins, glycosides and alkaloids and were effective against some strains of E. coli, P. aeruginosa and K. pneumoniae (Adebayo et al., 2001). Extracts of the leaves of Parkia biglobosa were reported.
Table 2. Diameters (mm) of zones of inhibitions produced by the aqueous and ethanolic extracts of *N. latifolia* (NL) and *D. oliveri* (DO).

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone diameter (mm)*</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>8.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>7.0</td>
<td>8.0</td>
<td>7.0</td>
<td>8.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>9.0</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
<td>9.0</td>
<td>8.0</td>
<td>7.0</td>
<td>8.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>10.0</td>
<td>11.0</td>
<td>11.0</td>
<td>10.0</td>
<td>9.0</td>
<td>10.0</td>
<td>9.0</td>
<td>9.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>12.0</td>
<td>9.0</td>
<td>9.0</td>
<td>9.0</td>
<td>10.0</td>
<td>6.0</td>
<td>7.0</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td></td>
<td>12.0</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
<td>10.0</td>
<td>11.0</td>
<td>8.0</td>
<td>9.0</td>
<td>6.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* 12.5 µg gentamicin zone diameters = *P. aeruginosa* 5 mm; *E. coli* 5 mm; *S. aureus* 6 mm; *K pneumoniae* 6 mm; *S. dysentriae* 8 mm.

Table 3. Minimum Inhibitory concentration (MIC) (mg/ml⁻¹) *N. latifolia* (NL) and *D. oliveri* (DO).

<table>
<thead>
<tr>
<th>Organisms</th>
<th><em>MIC (mg/ml⁻¹)</em></th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
<th>Leave</th>
<th>Bark</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
<td>Ethanol</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>100</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td></td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td></td>
<td>50</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

*Gentamycin (MIC) values for the test organisms = *P. aeruginosa* 25 µg/ml⁻¹; *K. pneumonia* 12.5 µg/ml⁻¹; *E. coli* 12.5 µg/ml⁻¹; *S. aureus* 12.5 µg/ml⁻¹; *S. dysentriae* 12.5 µg/ml⁻¹.

to contain tannins, alkaloids, steroids and cardiac glycol-side and were demonstrated to inhibit the growth of *B. cereus* and *S. aureus* (Ajaiyeoba, 2002; Isuzu and Harvey, 2003). The presence of these bioactive components in the crude drugs have been linked to their active-ties against disease causing microorganisms (Farnsworth, 1990) and also offering the plants them-selves protection against infection by pathogenic micro-organisms (De and Ifeoma, 2002).

The antibacterial properties of the extracts expressed in forms of diameter of zones of inhibition are given in Table 2. At a concentration of 100 g/ml⁻¹, the extracts, though less effective than 12.5 µg gentamicin, inhibited the growth of the pathogens, with *P. aeruginosa* and *E. coli* less susceptible similar to the findings of Ajaiyeoba (2002). The lack of susceptibility of *P. aeruginosa* and *E. coli* to the extracts could be attributed to the fact that these bacteria are inherently resistant to many antibiotics and non-antibiotic antimicrobial agents due to the perme-ability barrier afforded by their outer membranes (Lino and Deogracious, 2006). The aqueous extracts of the plants displayed smaller zones of inhibitions and the higher MIC values against all test organisms compared to ethanolic extract.

The MIC of the extracts obtained by doubling dilutions showed that *P. aeruginosa* has the highest MIC and therefore less susceptible. Contrary to the data presented by Lino and Degracious (2006), the ethanolic extracts are more potent in terms of activity than the aqueous extracts probably because the bioactive components are less soluble in water leading to incomplete extraction of the components than ethanol (De and Ifeoma, 2002). The higher solubility of tannins and phenols in ethanol might have contributed for the higher activity observed (Wolinsky and Sote 1982). Extracts from the roots are more effective than those from the bark and leaves, similar to the findings of Banso and Olatimayin (2001). The test organisms *E. coli*, *K. pneumonia*, *S. dysentriae* and *P. aeruginosa* are associated with both nosocomial and community acquired infections and is susceptible to the crude plant extracts. These pathogens are known to cause majority of community and hospital acquired infec-tions and are capable of elaborating several virulence factors including the formation of biofilms on colonized surfaces (Indrayan et al. 2002; De and James, 2002). This may explain why the leaves, bark, and stem are used as herbal recipe in the treatment of diarrhea and dysentery urinary tract infections and infections involving the buccal cavity. In an earlier study, Banso and Olutimayin (2001) demonstrated the activity of *D. oliveri*...
S. aureus, Streptococcus spp, E. coli and Clostridium perfringens. Gentamicin exhibited a higher activity than ethanolic extracts from both plants which in turn exhibited higher activity than aqueous extracts. This could be attributed to the fact that unlike conventional antibiotics and other pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin, which may be subjected to contamination and deterioration (Babu et al., 2002). The storage of extracts may require special condition of humidity, temperature and or protection from light. The plants extract might contain little of the active ingredient. The extracts which were inactive in vitro may have properties similar to prodrugs which are administered in an inactive form, but their metabolites could be active in-vitro (Lino and Deogracious, 2006).

In conclusion, water and ethanolic extracts of Nauclea latifolia and D. oliveri were assessed in this study. The results seem to justify their continued use in the treatment of microbial infections. The inhibition of growth of the test organisms that are known to cause nosocomial infections and displaying multidrug resistance to most antibiotics and non-antibiotic antimicrobial agents justify the continued use of these plants in folk and traditional medical practice. Studies should therefore be done in order to identify the active phytochemical constituents and evaluate their effectiveness in vitro so that they can be synthesized and commercial production begins in earnest.

ACKNOWLEDGEMENT

We acknowledge the contributions of Dr. Mbuh Florence for going through the manuscript and offering her suggestions.

REFERENCES


